DATA EVALUATION RECORD

ETHABOXAM
(LGC-30473)
[OPPTS (§83-3)]

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY IN THE RAT

MRID 46387808 (main study), 46488701, 46387806

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 103-2005E

Primary Reviewer: Stephanie J. Garcia, Ph.D.	Signature: Date:	Carol S Wood for S. J. Garcia AUG 15 2005
Secondary Reviewers: Carol Wood, Ph.D., D.A.B.T.	Signature: Date:	Carol S Wood AUG 15 2005
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross AUG 15 2005
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson AUG 15 2005

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EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** KJB
Registration Action Branch 2, Health Effects Division (7509C) **Date:** 4/25/06
EPA Work Assignment Manager: Ghazi Dannan, Ph.D. **Signature:** Ghazi Dannan
Registration Action Branch 3, Health Effects Division (7509C) **Date:** 4/25/06

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 090205 **DP BARCODE:** D313732

TEST MATERIAL (PURITY): Ethaboxam (LGC-30473, 97.5% a.i.)

SYNONYMS: *(RS)-N*-(α-cyano-2-thenyl)-4-ethyl-2-(ethylamino)-1,3-thiazole-5- carboxamide

CITATION: Gardner, T. (1997) LGC-30473: Repeat Study for Effects on Embryofoetal Development in the Rat by Gavage Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number LKY 58/963782, August 5, 1997. MRID 46387808. Unpublished.

Gardner, T. (1997) LGC-30473: Study for Effects on Embryofoetal Development in the Rat by Gavage Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number LKY 36/961722, August 5, 1997. MRID 46488701. Unpublished.

Gardner, T. (1996) LGC-30473: A Dose Range Finding Study in the Pregnant Rat by Gavage Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number LKY 35/961328, November 5, 1996. MRID 46387806. Unpublished.

SPONSOR: LG Chemical Ltd. (LG Life Sciences Ltd.), Taejon, KOREA.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46387808), Ethaboxam (LGC-30473, 97.5% a.i.) was administered to 25 female Crl:CD® BR VAF/Plus rats/dose by oral gavage at dose levels of 0, 10, 30, 100, or 300 mg/kg bw/day from gestation day (GD) 6 through 19, inclusive. The vehicle was 1% methylcellulose. On GD 20, all females were killed by CO_2 asphyxiation and subjected to macroscopic *post mortem* examination. Half of the fetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities; the remainder were fixed and stained for skeletal examination.

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Fur loss/alopecia was observed on seven animals at 100 mg/kg/day and eight animals at 300 mg/kg/day compared with one control animal. Absolute body weight was similar between the treated and control groups throughout the study. Treatment at 300 mg/kg/day was associated with transient reductions in maternal body weight gain (GD 6-8: control: 9.2 g; 300 mg/kg/day: 4.1 g; GD 6-12: control: 41.3 g; 300 mg/kg/day: 35.3 g) and food intake (91% of control for GD 6-7). Dose-related increased water consumption was observed for all treated groups: at 300 mg/kg/day, water consumption was up to 145% of control for 13 treatment days; at 100 mg/kg/day, water consumption was up to 124% of control for 8 treatment days; at 30 mg/kg/day, water consumption was up to 124% of control for 7 treatment days; and at 10 mg/kg/day, water consumption was up to 118% of control for 3 treatment days. Maternal necropsy was unremarkable.

The maternal toxicity LOAEL is 100 mg/kg bw/day based on hair loss and increased water consumption. The maternal NOAEL is 30 mg/kg bw/day.

The number of fetuses (litters) examined was 232 (18), 311(24), 268(23), 272(24), and 287(23) in the 0, 10, 30, 100, and 300 mg/kg/day groups, respectively. Pre-implantation loss for the 100- and 300-mg/kg/day groups was significantly greater than that of the control (13.5% and 11.6%, respectively, compared to 7.9% for the controls), but no effects were observed on the mean number of implantations or mean number of live fetuses. The distribution of dams showing total resorption (1 control and 2 at 30 mg/kg/day) did not suggest a relationship to treatment. The fetal sex ratio was unaffected by treatment. Fetal body weight was similar between the treated and control groups.

No treatment-related external or skeletal malformations/variations were observed. The incidence of litters containing fetuses with abnormal liver lobation was higher than that of the control group at 100 and 300 mg/kg/day. The number of fetuses (litters) with abnormal liver lobation was 2(2), 3(2), 2(2), 4(4), and 7(5), in the 0, 10, 30, 100, and 300 mg/kg/day groups, respectively. Increased incidences of abnormal liver lobation, and thin diaphragm with liver protrusion were also observed in another developmental study (MRID 46488701) at 100, 300, and 1000 mg/kg bw/day.

The developmental toxicity LOAEL is 100 mg/kg/day based on abnormal liver lobation. The developmental toxicity NOAEL is 30 mg/kg bw/day.

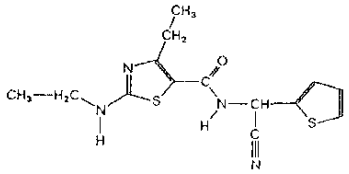
The developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3700; OECD 414) for a developmental toxicity study in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: LGC-30473

Description:	Fungicide, light brown powder (white crystalline powder, p. 89), store at 4°C in dark
Lot/Batch #:	4-1, received from sponsor (LG Chemical Ltd.) 10 April 1996
Purity:	97.5 % a.i.
Compound Stability:	Stable for duration of study; 8 days at 4°C in dark, 2 days under ambient conditions; No expiration date stated
CAS #of TGAI:	162650-77-3
Structure:	

2. Vehicle and/or positive control: 1% methylcellulose; Lot/Batch # and purity not stated.

3. Test animals:

Species:	Rats (females time-mated to males of the same strain)	
Strain:	Specific Pathogen Free (Crl: CD® BR VAF/Plus strain)	
Age/weight at study initiation:	Sexually mature; 8-10 weeks old; 172-248 g	
Source:	Charles River UK Ltd (Kent, England)	
Housing:	5/cage in suspended steel cages (Biotech) equipped with solid sides and wire grid front, back, floor and top; Cages constituting each treatment group were spatially dispersed to alleviate environmental influences	
Diet:	Special Diet Services (SDS) Laboratory Animal Diet No. 1 *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	18-22°C (transiently and infrequently outside this range)
	Humidity:	46-58% (transiently and infrequently outside this range)
	Air changes:	Not stated
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	3 days acclimation prior to allocation to treatment groups	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: 25 November 1996; End: 11 December 1996.

2. Mating: Sexually mature female rats were time-mated to males of the same strain prior to receipt from Charles River UK Ltd. (Margate, Kent). It was not specifically stated whether females were nulliparous nor whether mating of siblings was avoided. The first batch (A) consisted of 47 animals and was followed by a 2nd and 3rd batch (B and C) consisting of 42 animals each mated on consecutive days. The day of mating, as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug, was considered Day 0 of pregnancy.

3. Animal assignment: Allocation to treatment groups occurred on GD 3 when animals were weighed and assigned to 5 groups by computerized stratified randomization to give

approximately equal initial group mean body weight within each batch. Adjustments were made to the group allocation in order to ensure an acceptable distribution of females mated to the same male. Animals were assigned to dose groups as indicated in Table 1. Animals were earmarked for individual identification.

TABLE 1. Animal assignment

Dose (mg/kg bw/day)	0	10	30	100	300
# Females	25	25	25	25	25

4. **Dose selection rationale:** The dose levels were selected based on the results of a previous study of the same design performed at the testing facility (Appendix 1) as well as data from a range-finding study (Appendix 2). In the previous study, at doses of 100, 300, and 1000 mg/kg/day, a clear no effect level for embryofetal toxicity was not identified. The present study was undertaken with an identical protocol except that four treatment levels were chosen (10, 30, 100, and 300 mg/kg/day) in order to further assess the response to the test substance and to identify a clear no effect level for embryofetal toxicity.

5. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared weekly and stored at 4°C in the dark. Appropriate amounts of test substance (ground with a mortar and pestle) were mixed with 1% methylcellulose. A suspension was prepared for each treatment level by direct dilution and mixed using a Silverson mixer fitted with a fine screen. The analytical procedure validation, homogeneity, and stability of LGC-30473 in 1% methylcellulose was confirmed for concentrations at 1 mg/mL and 250 mg/mL during an earlier study (Report # LKY 35/961328; March-April 1996). Stability, concentration, and homogeneity (top, middle, and bottom) of the test substance in 1% methylcellulose were evaluated for a period of 0 (at 21°C) , 2 (at 21 or 4°C), and 8 days (at 4°C). On Day 1 of treatment in the current study, duplicate samples of freshly prepared test formulations were submitted for concentration analysis. Data for homogeneity and stability are presented from the earlier study.

Results:

Homogeneity analysis: At nominal concentrations of 1 and 250 mg/mL, LGC-30473 produces a homogenous suspension in 1% methylcellulose which can be maintained for up to 2 hours while magnetically stirred and successfully resuspended following ambient temperature storage for 2 days and refrigerated storage for 8 days (range under any of these conditions was 90-106% of nominal for concentrations at top, middle, and bottom).

Stability analysis: Stability analysis confirmed that the concentration remained close to nominal (± 8.5%) during storage in the dark at ambient temperature for 2 days and under refrigeration for 8 days.

Concentration analysis: The mean concentration of LGC-30473 in test formulations analyzed during the study were within ± 8% of nominal concentrations [for nominal concentrations of 1, 3, 10, and 30 mg/mL, actual concentrations were 0.928 (92.8%), 2.90 (96.7%), 9.76 (97.6%), and 28.4 (94.7%) mg/mL, respectively].

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The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered once daily by oral gavage on GD 6 through 19, inclusive, in a volume of 1 mL/100 g of body weight/day, calculated to the nearest 0.1 mL. Control animals received the vehicle alone at the same dose volume. Dosing was adjusted according to body weight on GDs 8, 10, 12, 14, 16 and 18. Animals were dosed at approximately the same time each day, where possible.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked daily for mortality or clinical signs. Body weight data were recorded initially on arrival (GD1 for batches A and C, GD2 for batch B) and on GDs 3, 6, 8, 10, 12, 14, 16, 18, and 20. Food consumption was measured from weighday to weighday commencing on GD3. Water consumption was recorded daily from GD3. Dams were sacrificed on GD20 via CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined immediately to determine: number of corpora lutea, number and distribution of live young and embryofetal deaths, individual fetal weight from which litter weight is calculated, gross fetal abnormalities, and gravid uterine weight. Embryonic/fetal deaths were classified as early (only placenta visible at termination) or late (both placental and embryonic remnants visible at termination). Uteri or individual uterine horns without visible implantations were examined for evidence of implantation using a modified Salewski technique.

2. **Fetal evaluations**: Live fetuses were examined externally and weighed. Half of the fetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique); the remainder were fixed in 74 OP industrial methylated spirit for subsequent macroscopic examination, evisceration, clearing and alizarin staining (modified Dawson technique) for skeletal examination. Fetuses showing suspected abnormalities were processed further by the more appropriate technique for clarification of initial observations. Fetuses were uniquely identified to allow correlation of initial with subsequent findings. All fetuses were sexed by gonadal inspection following preservation.

   Structural changes were presented as malformations, anomalies, or variants as defined below:
   Malformations: Rare and/or probably lethal (e.g., exencephaly, anury).
   Anomalies: Minor differences from 'normal' that are detected relatively frequently either by free-hand sectioning (e.g., increased renal pelvic dilation), or at skeletal examination (e.g., bipartite centrum).
   Variants: Alternative structures occurring regularly in the control population (e.g., ossified sternebra(e)).

D. DATA ANALYSIS:

1. **Statistical analyses:** Significance tests, employing analysis of variance (ANOVA) followed by an intergroup comparison with control, were performed on the following parameters: bodyweight change, food and water consumption, litter data and fetal abnormalities.

   Dependent on the heterogeneity of variance between treatment groups, parametric tests (ANOVA) followed by Williams' test or non-parametric tests (Kruskal-Wallis) followed by Shirley's test were used to analyze the data, as appropriate. Food and water consumption data were analyzed on a cage basis; bodyweight change analysis used the individual animal as the basic experimental unit. For mean litter data and fetal variants, the litter was the unit of statistical analysis; due to the preponderance of non-normal distributions, non-parametric analyses were routinely used. Analysis of the incidence and distribution within litters of pre-implantation loss, *in utero* deaths, and fetal malformations and anomalies was performed using the Linear by Linear Association test in a step-down fashion. Where the Linear by Linear Association test was not statistically significant, the Kruskal-Wallis test was used to detect non-linear responses. If the Kruskal-Wallis test was significant (at 1% level), then pair-wise permutation tests were used to compare each dose level with the control. Where 75% or more of the values for a given variable were the same, Fisher's exact test was used.

   All significant ($p \leq 0.05$) intergroup differences from the control are reported and were supported by a significant ANOVA ($p \leq 0.05$), unless otherwise indicated.

   The reviewer considers the statistical analyses to be appropriate.

2. **Indices:** The following indices were calculated by the reviewer from cesarean section records of animals in the study:

   Corrected Body Weight (BW) Gain = $[BW_{GD\ 20} - BW_{GD\ 6}] - [\text{Uterus Weight}_{GD\ 20}]$.

   Pre-implantation loss (%) = [(# corpora lutea - # implants)/# corpora lutea] × 100.

   Post-implantation loss (%) = [(# implants - # live fetuses)/# implants] × 100.

3. **Historical control data:** Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** All animals survived until scheduled termination. Yellow stained undercage tray paper was noted from the second day of dosing for all cages of animals receiving 100 or 300 mg/kg/day, for up to four days or throughout the treatment period respectively. One cage of animals receiving 30 mg/kg/day showed this sign on the second day of treatment only.

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An increased incidence of dorsal hair loss or alopecia appearing during the treatment period and persisting to termination was noted among animals receiving 100 and 300 mg/kg/day compared to control and lower dosage animals (incidence of hair loss: 1, control; 2, 10 mg/kg/day; 2, 30 mg/kg/day; 7, 100 mg/kg/day; 8, 300 mg/kg/day).

2. **Body weight:** Body weight data are summarized in Table 2. Absolute body weight was similar between the treated and control groups throughout the study. Group mean body weight gain at 300 mg/kg/day was significantly less than that of controls for GD 6-8 and GD 6-12 ($p < 0.05$ or 0.01). Subsequent maternal weight gain throughout the treatment period was similar to the control group. Group mean weight gain for animals receiving 10, 30, or 100 mg/kg/day was similar to controls.

TABLE 2. Mean (± SD) maternal body weight and body weight gain (g) [a]

Day/Interval	Dose in mg/kg bw/day (# of Dams)				
	0 (18)	10 (24)	30 (23)	100 (24)	300 (23)
Body weight GD 6	249.1	249.0	251.6	245.8	246.0
Body weight GD 8	258.3	260.2	261.9	255.3	250.1
Body weight GD 12	290.4	291.0	292.3	285.0	281.2
Body weight GD 16	317.3	318.6	320.3	311.1	309.7
Body weight GD 20	377.3	380.8	378.6	368.2	370.0
Weight gain GDs 3-6	18.1	19.2	20.1	18.3	17.6
Weight gain GDs 6-8	9.2	11.3	10.3	9.4	4.1** (45)
Weight gain GDs 6-12	41.3	42.0	40.7	39.1	35.3* (85)
Weight gain GDs 6-16	68.2	69.7	68.7	65.3	63.7
Weight gain GDs 6-20	128.2	131.8	127.0	122.3	124.0
Corrected BW Gain [b]	55.6 ± 17.0	59.8 ± 12.4	52.1 ± 21.6	58.0 ± 14.0	56.4 ± 13.0

[a] Data obtained from Table 2, p. 25 and Appendix 5, pp. 48-52, MRID 46387808. Standard deviations were not calculated.
[b] Corrected body weight gain calculated by reviewer from individual data according to formula in section D.2.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

3. **Food and water consumption:** Food and water consumption values attaining statistical significance are presented in Table 3. During the first two days of treatment, group mean food consumption at 300 mg/kg/day was significantly reduced compared to concurrent controls. Henceforth, and throughout the treatment period for animals treated with 10, 30, or 100 mg/kg/day, variations in food consumption (sometimes attaining statistical significance compared with concurrent control values) were considered incidental to treatment with LGC-30473.

Group mean water consumption was significantly increased compared to controls by animals treated with 300 mg/kg/day during the treatment period. Statistically significant increases also were noted on most days from Day 11 for females treated at 30 or 100 mg/kg/day. Values recorded for animals treated at 10 mg/kg/day were marginally higher than controls, completing a generally dose-related response for this parameter.

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TABLE 3. Mean ± SD food and water consumption (g/rat/day) [a]					
Interval/ day	Dose in mg/kg bw/day (# of Dams)				
	0 (18)	10 (24)	30 (23)	100 (24)	300 (23)
Food consumption					
6-7	22 ± 1 [b]	23 ± 1	23 ± 1	22 ± 1	20 ± 1** (91%)
12-13	23 ± 2	26 ± 1** (113%)	26 ± 1** (113%)	25 ± 1** (109%)	25 ± 1** (109%)
16-17	25 ± 3	29 ± 2* (116%)	29 ± 2* (116%)	29 ± 1* (116%)	28 ± 2* (112%)
18-19	25 ± 3	29 ± 2* (116%)	29 ± 1* (116%)	28 ± 2* (112%)	28 ± 1* (112%)
Water consumption					
7	27 ± 3	27 ± 2	30 ± 2	29 ± 1	31 ± 2 (115%)*
8	26 ± 3	27 ± 4	27 ± 4	27 ± 3	33 ± 4 (127%)**
9	29 ± 5	29 ± 3	32 ± 3	32 ± 2	37 ± 7 (128%)**
10	29 ± 4	29 ± 4	31 ± 1	30 ± 2	39± 10 (134%)**
11	31 ± 3	32 ± 2	34 ± 3	35 ± 2 (113%)*	39 ± 4 (126%)**
12	28 ± 4	32 ± 1 (114%)*	33 ± 2 (118%)**	32 ± 2 (114%)**	35 ± 2 (125%)**
13	29 ± 3	32 ± 2	35 ± 2 (121%)*	33 ± 4 (114%)*	38 ± 4 (131%)**
14	30 ± 5	32 ± 5	34 ± 2	33 ± 4	38 ± 3 (127%)**
15	32 ± 3	35 ± 3	38 ± 2 (119%)**	38 ± 2 (119%)**	43 ± 4 (134%)**
16	32 ± 4	37 ± 3 (116%)*	39 ± 3 (122%)**	39 ± 1 (122%)**	44 ± 4 (138%)**
17	35 ± 2	38 ± 5	42 ± 2 (120%)*	41 ± 0.4 (117%)*	47 ± 4 (134%)**
18	34 ± 4	38 ± 4	40 ± 2 (118%)*	40 ± 3 (118%)**	48 ± 3 (141%)**
19	33 ± 5	39 ± 4 (118%)*	41 ± 4 (124%)**	41 ± 2 (124%)**	48 ± 4 (145%)**

[a] Data obtained from pp. 26-27 (MRID 46387808).
[b] Standard deviation calculated by Reviewer from individual data on pp. 45-47 (MRID 46387808).
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

4. **Gross pathology:** Macroscopic *post mortem* examination of dams on GD 20 confirmed dorsal alopecia on 7 and 8 females in the 100 or 300 mg/kg/day groups, respectively, compared with one similarly affected control animal.

5. **Cesarean section data:** Data are summarized in Table 4. The control group had an unusually low pregnancy rate, the reason for this is unknown. Pre-implantation loss for the 100- and 300-mg/kg/day groups was significantly greater than that of the control, but no effects were observed on the mean number of implantations or mean number of live fetuses. The distribution of dams showing total resorption (1 control and 2 at 30 mg/kg/day) did not suggest a relationship to treatment. The sex ratio was unaffected by treatment. Fetal body weight was similar between the treated and control groups.

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TABLE 4. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)				
	0	10	30	100	300
# Animals assigned (mated)	25	25	25	25	25
# Animals pregnant	19	24	25	24	23
Pregnancy rate (%)	76	96	100	96	92
# Nonpregnant	6	1	0	1	2
Maternal wastage					
# Died	0	0	0	0	0
# Aborted	0	0	0	0	0
# Premature delivery	0	0	0	0	0
Total # corpora lutea Corpora lutea/dam	265 14.7 ± 2.4	338 14.1 ± 2.1	301 13.1 ± 3.2	326 13.6 ± 3.0	335 14.6 ± 3.1
Total # implantations Implantations/dam	244 13.6 ± 1.7	322 13.4 ± 2.0	281 12.2 ± 3.3	282 11.8 ± 3.6	296 12.9 ± 3.6
Total # litters	18	24	23	24	23
Total # live fetuses Live fetuses/dam	232 12.9 ± 1.6	311 13.0 ± 2.3	268 11.7 ± 3.2	272 11.3 ± 3.6	287 12.5 ± 3.5
Total # dead fetuses	0	0	0	0	0
Total # resorptions	12	11	13	10	9
Early	12	10	13	8	9
Late	0	1	0	2	0
Resorptions /dam	0.7	0.5	0.6	0.4	0.4
Early	0.7	0.4	0.6	0.3	0.4
Late	0.0	0.0	0.0	0.1	0.0
Litters with total resorptions	1	0	2	0	0
Mean litter weight (g)	47.7 ± 6.4	47.6 ± 8.0	44.4 ± 12.2	42.1 ± 13.3	44.4 ± 12.2
Mean fetal weight (g)	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.4	3.7 ± 0.2	3.6 ± 0.3
Mean gravid uterine weight (g)	72.6 ± 9.0	72.1 ± 11.4	67.8 ± 18.6	64.3 ± 19.2	67.6 ± 17.5

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Observation	Dose (mg/kg bw/day)				
	0	10	30	100	300
Sex ratio (% male)	52.8	47.3	49.6	52.9	53.9
Preimplantation loss (%)	7.9	4.7	6.6	13.5**	11.6*
Postimplantation loss (%)	4.9	3.4	4.6	3.5	3.0

[a] Data obtained from Tables 1 and 5, pp. 24 and 28, respectively, and Appendix 5, pp. 48-52, MRID 46387808. Only mean values were presented in the summary tables. Group totals and standard deviations (where applicable) were calculated by the reviewer from individual litter data.
* Statistically different ($p < 0.05$) from the control.

B. DEVELOPMENTAL TOXICITY: The number of fetuses (litters) examined was 232(18), 311(24), 268(23), 272(24), and 287(23) in the 0, 10, 30, 100, and 300 mg/kg/day groups, respectively.

1. **External examination:** One fetus from a control litter had forelimb flexure with oligodactyly and syndactyly. No other information regarding external examination was presented.

2. **Visceral examination:** The incidence of litters containing fetuses with abnormal liver lobation was higher than that of the control group at 100 and 300 mg/kg/day (Table 5). The author noted that the incidence at 100 mg/kg/day was within the background range while that at 300 mg/kg/day was slightly above the highest incidence seen in control groups from recent studies. However, the historical control data were not given.

   A higher incidence of fetuses with hemorrhage affecting the brain was noted at 300 mg/kg/day, just above that seen in control groups from other recent studies (data not included). This finding was not considered by the author to be an effect of treatment since it can occur as an artifact of handling of the fetuses.

3. **Skeletal examination:** No treatment-related skeletal malformations/variations were observed in any fetus (Table 5). While it was noted that all treated groups showed a higher incidence of irregular ossification of thoracic vertebral centra than the control group, this finding was considered unrelated to treatment as the control value was low and the incidence at 300 mg/kg/day was within the recent historical control range (2 fetuses in 2 litters up to 8 fetuses in 8 litters). The data also lacked a dose-response relationship.

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TABLE 5. Visceral and skeletal examinations [a]					
Observations [b]	Dose (mg/kg bw/day)				
	0	10	30	100	300
Total # fetuses(litters) examined	232 (18)	311 (24)	268 (23)	272 (24)	287 (23)
#Fetuses(litters) examined viscerally	113 (18)	153 (24)	134 (22)	136 (24)	141 (23)
#Fetuses(litters) examined skeletally	116 (18)	157 (24)	134 (23)	135 (24)	142 (22)
#Fetuses(litters) affected-malformations	3 (3)	1 (1)	0	1 (1)	4 (3)
Visceral examination					
#Fetuses(litters) affected-visceral anomaly [c]	20 (9)	19 (15)	19 (10)	24 (17)	22 (16)
Anury and non-patent anus	0	0	0	0	2 (1)
Abnormal liver lobation	2 (2)	3 (2)	2 (2)	4 (4)	7 (5)
Hemorrhage affecting brain	1 (1)	3 (3)	3 (2)	2 (1)	6 (5)
Dilated renal pelvis/ureter	4 (2)	1 (1)	3 (3)	2 (2)	2 (2)
Displaced testis(es)	3 (3)	3 (3)	2 (2)	6 (6)	3 (3)
Skeletal examination					
#Fetuses (litters) affected-skeletal anomaly [c]	14 (9)	9 (8)	19 (13)	14 (9)	17 (10)
Irregular ossification vertebral centra	2 (2)	6 (5)	11 (9)	8 (7)	8 (6)
Complete lumbar rib(s)	0	0	0	0	4 (2)
Sutural bone	1 (1)	1 (1)	3 (3)	0	0
Incomplete ossification of:					
One or more cranial centers	4 (4)	1 (1)	4 (2)	0	3 (3)
Cervical vertebral arches	2 (2)	1 (1)	2 (2)	1 (1)	1 (1)
Sacrocaudal vertebral arches	5 (4)	2 (2)	5 (4)	4 (3)	2 (1)
One or more centers pelvic girdle	3 (3)	1 (1)	2 (1)	4 (3)	1 (1)
Digital centers	1 (1)	0	1 (1)	1 (1)	0

[a] Data obtained from Tables 7-9, pp. 30-32, respectively, MRID 46387808.
[b] Individual fetuses may occur in more than one category.
[c] Excludes malformed fetuses.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Excretion of considerable amounts of the parent compound and/or metabolites at 100 and 300 mg/kg/day was suggested by the non-specific sign of yellow stained tray paper. This has been noted in previous studies with LGC-30473.

The 300 mg/kg/day dose was maternally toxic, producing initial impairment of body weight gain and food intake, with increased water consumption and fur loss also noted. The slightly reduced fetal weight was close to the low end of the expected normal range and was considered unlikely to be of biological significance as an isolated finding. Thus there were only equivocal suggestions of fetal toxicity at 300 mg/kg/day in this study.

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Treatment at 100 mg/kg/day was associated with minor effects on maternal water consumption and fur loss but no apparent effects on the developing fetus.

Slightly higher maternal water consumption was associated with treatment at 30 and 10 mg/kg/day, without adverse effects on *in utero* development of the offspring.

In the context of this study alone, it was conceivable that 100 mg/kg/day was a NOAEL for embryofetal toxicity. However, taking the findings of the first study (Appendix 1) into consideration, there was some equivocal evidence of marginal retardation of embryofetal development at 300 and 100 mg/kg/day, with changes observed that were not considered to have been caused by the mild degree of maternal toxicity. It could only be confidently concluded that 30 mg/kg/day was a clear NOAEL for embryofetal development, as identified in the present study.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Clinical signs of toxicity were limited to the 100 and 300 mg/kg/day groups and consisted of yellow staining under the cages and alopecia. Yellow staining is most likely due to excretion of the test article or a metabolite and is not considered adverse. In contrast, alopecia has been seen in other studies with LGC-30473 and is considered adverse although the mechanism is unknown. Treatment at 300 mg/kg/day was associated with a transient reduction in maternal body weight gain and food intake for several days after the initiation of treatment. The slightly lower weight gain by animals at 300 mg/kg/day did not result in any effects on absolute body weight. Dose-related increases in water consumption were observed for all treated groups. It is unknown whether the increased water consumption was due to an irritant property of the chemical, bad taste, or some other reason. In the reviewer's opinion, the significant ($p \leq 0.01$ or $p \leq 0.05$) increases in water consumption (increased 113-145%) cannot be dismissed, and coupled with the increased incidences of alopecia (7 vs 1 in controls) is considered treatment-related and possibly adverse.

   The maternal toxicity LOAEL is 100 mg/kg bw/day based on hair loss and increased water consumption. The maternal NOAEL is 30 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** The distribution of dams showing total resorption (control: 1; 30 mg/kg/day: 2) did not suggest a relationship to treatment. Post-implantation loss was not affected by treatment and no dead fetuses were observed at cesarean section.

   b. **Altered growth:** No indication of altered fetal growth was observed in the current study. The reviewer disagrees with the study author and thinks that a mean fetal body weight of 3.6 g at 300 mg/kg/day is not different from 3.7 g for the controls. These results are in contrast to the findings in a previous study (Appendix 1) in which fetal body weight appeared to be reduced at maternal doses of ≥ 100 mg/kg/day. However, examination of the data from the previous study revealed no differences between the 100- and 300-mg/kg/day groups (mean fetal weight 3.48 g and 3.51 g, respectively) and a relatively

high control value (3.78 g) with clear effects at 1000 mg/kg/day (3.00 g). Similar results for fetal body weight were found in the range-finding study (Appendix 2). Historical control data for fetal body weight at the testing facility is needed before conclusions can be made regarding the effects of the test article on fetal growth. In addition, ossification rates for fetuses in the current study were similar between the treated and control groups.

c. **Developmental variations and anomalies:** Although not statistically significant, there was some evidence for increased incidences of visceral anomalies including abnormal liver lobation at 100 and 300 mg/kg/day and brain hemorrhage at 300 mg/kg/day. In a previous study (Appendix 1), a slight increase in abnormal liver lobation was also seen at doses of ≥ 100 mg/kg/day and was accompanied by diaphragmatic hernia or thin diaphragm with liver protrusion at 1000 mg/kg/day. While the relevance of abnormal liver lobation without concurrent diaphragm malformations is unclear, the reviewer does not think that it can be dismissed in the absence of historical control data. The reviewer agrees with the study author that brain hemorrhage can occur as an artifact of fetal processing and handling and may not be treatment-related. No clear, dose-related effect was seen for any skeletal variation; lumbar ribs and irregular ossification of the vertebral centra are common findings in rat fetuses.

d. **Malformations:** Four fetuses (in 3 litters) had malformations in the highest dose group compared to 3 fetuses (in 3 litters) in the control group. Thus the number of fetuses(litters) with malformations did not change with treatment at 300 mg/kg/day. The malformations observed were varied and no specific finding was increased.

The developmental toxicity LOAEL is 100 mg/kg/day based on abnormal liver lobation. The developmental toxicity NOAEL is 30 mg/kg bw/day.

C. **STUDY DEFICIENCIES:** Some minor deficiencies include: lack of historical control data; only 18 control litters in the main study instead of the required 20 litters; no stated expiration date for the test substance; varying descriptions of test material (light brown powder; white crystalline powder); and number of air changes not stated. The historical control data is needed to fully assess the effects on the fetus.

APPENDIX 1: Study for Effects on Embryofetal Development in the Rat by Oral Gavage

TEST MATERIAL (PURITY): Ethaboxam (LGC-30473; 97.5% a.i.)

CITATION: Gardner, T. (1997) LGC-30473: Study for Effects on Embryofoetal Development in the Rat by Gavage Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number LKY 36/961722, August 5, 1997. MRID 46488701. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46488701) LGC-30473 (97.5% a.i., batch 4-1) was administered to 25 time-mated Crl:CD® BR VAF/Plus rats/dose by oral gavage at dose levels of 0, 100, 300 or 1000 mg/kg bw/day from GD 6-19, inclusive. On GD 20, all dams were killed by CO_2 asphyxiation and subjected to macroscopic *post mortem* examination. The following observations were recorded: pregnancy status, number of corpora lutea, number and distribution of live young and embryofetal deaths, individual fetal weights, and gross fetal abnormalities. Half of the fetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities; the remainder were fixed and stained for skeletal examination.

All animals survived to scheduled sacrifice. Fur loss or alopecia was observed on 5, 9, 10, and 25 animals in the control, low-, mid-, and high-dose groups, respectively. Post-dosing salivation was seen on 3 mid-dose and 9 high-dose animals compared with none of the controls. Yellow staining was noted under the cages of the mid- and high-dose rats. No other treatment-related clinical signs were observed. Cumulative body weight gain by the high-dose group was reduced to 28-63% ($p \leq 0.01$ for all intervals) of the control level throughout the treatment period resulting in final body weight of the high-dose group 85% of the control level. The only other effect on body weight or body weight gain was a transient decrease in weight gain by the mid-dose group for GD 6-12 (88% of control; $p \leq 0.05$). Food consumption by the high-dose group was 69-85% of the controls for GDs 6-11 and 18-19. Water consumption was significantly increased compared to the controls beginning on GD 8 for the mid-dose group and on GD 7 for the high-dose group.

The maternal toxicity LOAEL is 300 mg/kg bw/day based on lower body weight gain, increased water consumption and post dose salivation. The maternal NOAEL is 100 mg/kg bw/day.

Three high-dose and one control dam had complete litter resorption. The mean number of corpora lutea, implantations, and live fetuses and the fetal sex ratio were similar between the treated and control groups. Fetal body weight for all treated groups was significantly less ($p \leq 0.01$) than that of the control group; mean fetal weight for the control, low-, mid-, and high-dose groups was 3.78 g, 3.48 g, 3.51 g, and 3.00 g, respectively. Only the effect at the high dose is considered treatment-related.

The number of fetuses (litters) examined in the control, low-, mid-, and high-dose groups was 269 (22), 276 (22), 284 (24), and 261 (21), respectively. In the high-dose group, six fetuses from 2 litters had misshapen pituitary and seven fetuses from four litters had diaphragmatic hernia compared with none of the controls. Other treatment-related findings in the control, low-, mid-,

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and high-dose fetuses(litters) included abnormal liver lobation in 1(1), 5(3), 5(5), and 9(7), respectively, and thin diaphragm with protrusion of the liver in 2(2), 2(1), 2(2), and 6(4), respectively. An increase in the incidence of incomplete or irregular ossification of the pelvic girdle, digits, sternebrae, and thoracic vertebral centra was found in the high-dose group compared to the control group.

The developmental toxicity LOAEL for LGC-30473 in rats is 100 mg/kg bw/day based on abnormal liver lobation. The developmental toxicity NOAEL is not determined.

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APPENDIX 2: A Dose Range Finding Study in the Pregnant Rat by Oral Gavage.

TEST MATERIAL (PURITY): Ethaboxam (LGC-30473; 97.5% a.i.)

CITATION: Gardner, T. (1996) LGC-30473: A Dose Range Finding Study in the Pregnant Rat by Gavage Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number LKY 35/961328, November 5, 1996. MRID 46387806. Unpublished.

EXECUTIVE SUMMARY: In a range finding oral developmental toxicity study (MRID 46387806), Ethaboxam (LGC-30473; 90.4% a.i.) was administered to groups of 10 female Crl:CD® BR VAF/Plus rats/dose in 1% methylcellulose by oral gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/d from days 6 through 19 of gestation, inclusive. On GD 20, the females were killed by CO_2 asphyxiation, dissected and subjected to macroscopic *post mortem* examination. The following observations were recorded: pregnancy status, number of corpora lutea, number and distribution of live young and embryofetal deaths, individual fetal weights, and gross fetal abnormalities. Fetuses were not examined for skeletal or visceral abnormalities.

All animals survived to scheduled sacrifice. The only clinical sign of toxicity was post-dosing salivation observed on 0, 4, 5, and 7 animals of the control, low-, mid-, and high-dose groups, respectively. Yellow staining was observed under the cage of all treated groups. Transient effects on body weight gain were seen in the high-dose group as weight loss for GD 6-8 and in the mid-dose group as weight gain 82% and 74% of the control levels for GDs 6-8 and 6-10, respectively. Food consumption by the high-dose group was 67-76% of the control levels for GDs 6-9. Water consumption (measured beginning on GD 13) was increased for the high-dose group. Gross necropsy was unremarkable.

The maternal toxicity LOAEL is 300 mg/kg bw/day based on reduced body weight gain. The maternal NOAEL is 100 mg/kg bw/day.

There were no complete litter resorptions. The mid-dose group had a decrease in the number of corpora lutea and implantations, an increase in post-implantation loss, and a corresponding decrease in live fetuses; the low- and high-dose groups were similar to the control group for these endpoints. Mean fetal body weight for the control, low-, mid-, and high-dose groups was 3.63 g, 3.54 g, 3.56 g, and 3.15 g, respectively. Only the effect at the high dose is considered treatment-related.

The number of fetuses (litters) examined in the control, low-, mid-, and high-dose groups was 98 (9), 99 (7), 33 (6), and 90 (8), respectively. No treatment-related external malformations/variations were found.

The developmental toxicity LOAEL is 1000 mg/kg bw/day based on reduced fetal body weights. The developmental toxicity NOAEL is 300 mg/kg bw/day.

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